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ELECTRODES FOR FUNCTIONAL NEUROMUSCULAR STIMULATION

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Abstract

A series of in vivo tests in rats has been performed to test the biological response to alternative silicone rubber and fluoropolymer materials used in the manufacture of electrodes. Histological evaluation of the encapsulation tissue surrounding the wire and cuff implants has been completed and is reported here. No difference in cellular response to the insulation materials was noted between the two fluoropolymers. The cellular response to the alternative silicone rubber materials was improved as compared to the response to the previously used material. Based on these results, we are comfortable using these materials in future studies and judge them suitable for continued investigation. Histological evaluation of neural tissue at the site of these cuff implants is ongoing.

Background

Silicone rubber nerve cuff electrodes were implanted on the sciatic nerves of adult rats. Segments of fluoropolymer insulated wire were placed subcutaneously on the backs of these same animals. Original and replacement materials were used in the manufacture of these implants. Two and 4 weeks after implantation, the animals were killed by aortic perfusion and the tissue was fixed for histological processing. Encapsulation tissue surrounding the implants was prepared for H&E and Trichrome staining; stained sections were evaluated using light microscopy. Neural tissue at the site of each cuff implant was prepared for Methylene Blue staining; the stained sections are currently undergoing evaluation through both light and electron microscopy.

Encapsulation Tissue: General Histology Results

Although the implant specimens were of multiple configurations, materials, and implant duration, some general observations of the cellular response across all sample groups were made and are discussed below.

Consistent with our expectations, the cellular response was noted to be generally decreased in those samples implanted for the longer time duration. Relative to the 2 week samples, the capsules surrounding the 4 week implants showed signs of maturation, with generally fewer inflammatory cells, increased amounts of collagen, improved organization of the collagen layers, and increased vascularization.

The capsules surrounding the implants were found to consist of anywhere from 1-2 up to 5 or more layers of round active cells, primarily macrophages and fibroblasts, lying at the capsule edge directly against the implant material. The round character of these cells is indicative of their high metabolic state, increased volume of cytoplasm and general increase in

activity. Beyond this active cell region, the capsule contained primarily elongated or elongating fibroblasts with developing collagen. The elongation of these cells, involving a general reduction in the volume of cytoplasm and decrease in metabolic activity, is a characteristic of the stable state of the cell, when it produces and maintains its associated collagen. Varied thickness, maturation, and organization of this collagen was found. The primary cell types within the capsule were macrophages and fibroblasts, although occasional polymorphonuclear cells and foreign body giant cells were also observed.

For all implant types, we noticed a relatively high degree of variability in the cellular response within a single implant group and oftentimes, even within a single sample. In these cases, one region of the capsule might consist of 1-2 round, active cell layers and then another region, sometimes directly adjacent or directly across from the first region, might consist of 5-10 layers of round active cells.

Vascularization within the capsule region was frequently present, although was more notable in the 4 week implant groups. The vessels were typically found several cell layers from the capsule edge. In a few instances, micro-hemorrhages were seen, with red blood cells present outside of the vasculature and spilled into the tissue. However, no evidence of prior hemorrhage or blood breakdown products were found in any sample. It is believed that these micro-hemorrhages were the result of mechanical irritation and likely occurred at the time of explant.

Encapsulation Tissue: Wire Implants

The wire implants in this study are divided into 18 different sample groups, each with n=3. The groups were determined by all combinations of time duration (2 or 4 weeks), insulation material (FEP or PFA fluoropolymer), sample length (short (1 cm) or long (7 cm)), and wire configuration (uncoiled or coiled). Additionally, long (7 cm) samples of closed helix lead segments (coiled wire inside silicone rubber tubing) were implanted for both time durations, and comprised the remaining 2 groups.

<u>General Observations</u>

As was described in the preceding section, a high degree of variability in cellular response was observed even within the same sample group. The photomicrographs presented below demonstrate the range of cellular response observed across all wire implants.

Wire Implants: Range of Response

Figure 1: Photomicrograph of tissue capsule surrounding a long, uncoiled segment of PFA insulated wire that was implanted for 2 weeks. (R9 #0101, H&E stain, original magnification=500x)

Figure 1 is an example of what could be considered a low level cellular response. The capsule directly abutting the wire (right side) consists of solely elongated fibroblasts, with essentially no round, active inflammatory cells. A loose collagenous matrix extends beyond the immediate capsule and at the very far edge contains several blood vessels.

Wire Implants: Range of Response

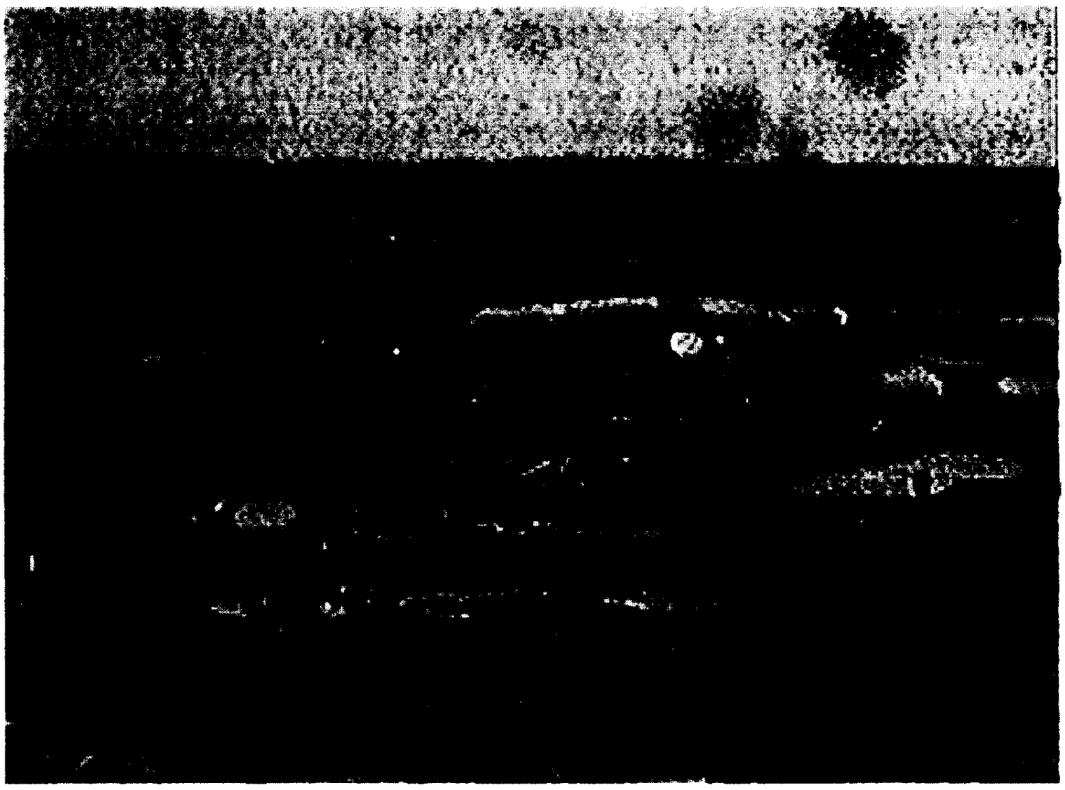

Figure 2: Photomicrograph of tissue capsule surrounding a short, uncoiled segment of FEP insulated wire that was implanted for 4 weeks. (R13 #1117, H&E stain, original magnification=500x).

A more typical response observed in these studies is presented in the figure above. The capsule here consists of 1-3 layers of round, highly metabolic cells at the edge abutting the implant material. Underlying layers of collagen and fibroblasts, as well as several blood vessels, are present in the peripheral regions of the capsule (lower edge of photomicrograph).

Wire Implants: Range of Response

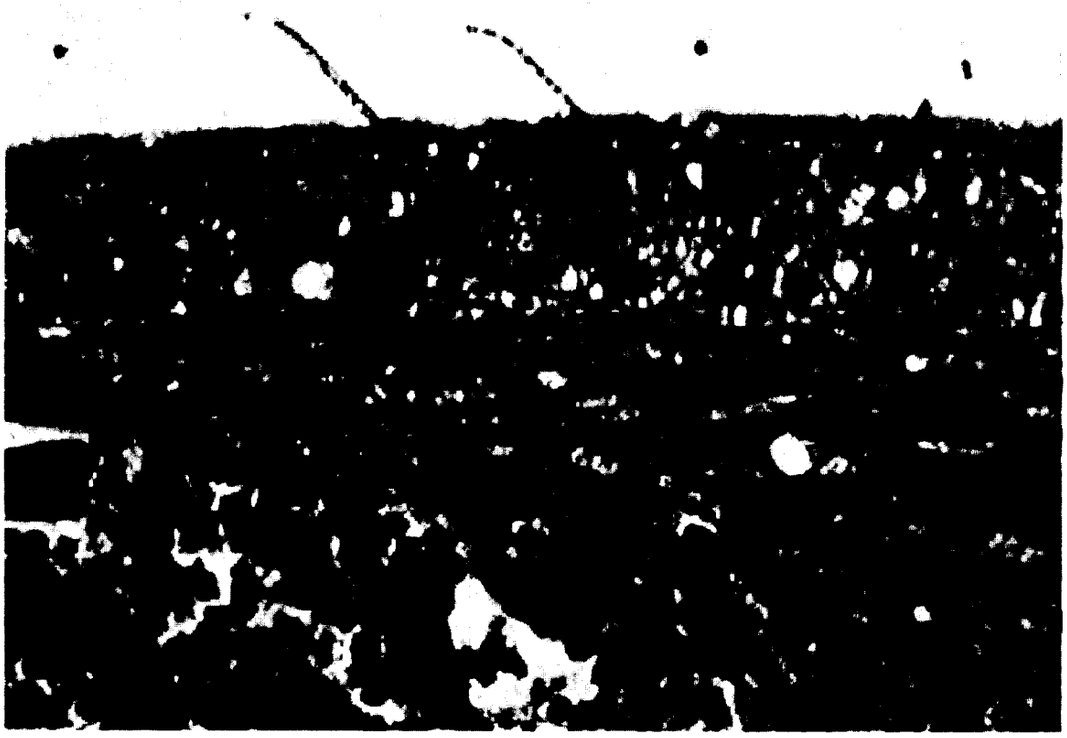

Figure 3: Photomicrograph of tissue capsule surrounding a long, coiled segment of FEP insulated wire that was implanted for 4 weeks. (R5 #1004, H&E stain, original magnification=500x)

Figure 3 is an example of a slightly elevated cellular response, as indicated by the increasing numbers of rounded cells at the capsule edge. Within the 3-4 active cell layers present we see very rounded cells, with increased cytoplasm and decreased capsular organization. Below this active cell region are elongating fibroblasts, collagen, and some blood vessels. In the lower left corner of the photo are numerous red blood cells spilled into the tissue, indicating a micro-hemorrhage.

Wire Implants: Range of Response

Figure 4: Photomicrograph of tissue capsule surrounding a long, coiled segment of FEP insulated wire that was implanted for 4 weeks. (R4 #1007, H&E stain, original magnification=500x)

Finally, in Figure 4, is an example of the most extensive cellular response observed in these implants. The capsule contains almost exclusively rounded cells, indicating an active cellular response, with very little organization. Only at the very edge of the photo, 8-10 cell layers from the capsule edge and the implant material, do we find elongating fibroblasts and any significant collagen.

The results presented in the preceding 4 figures are representative of what was seen in all sample groups and is indicative of the variability in cellular response found in this study. Comparisons were made between corresponding sample groups to investigate any trends in cellularity and to determine the impact of differences in implant duration, configuration, length and material. These results are summarized below.

Wire Implant Duration

A generally decreased cellular response was observed in those samples implanted for 4 weeks, as compared to those implanted for 2 weeks. This was true for all sample configurations and materials. This result was not surprising, as we would expect the acute inflammatory response, characterized by high numbers of macrophages and polymorphonuclear cells, to be resolving. These acute inflammatory cells are expected to be replaced by fibroblasts and developing collagen and vasculature, which characterize the maturation of the capsule.

Wire Implant Configuration

The cellular response surrounding the straight wire configurations, including both the uncoiled wires and the closed helix leads, and the cellular response at the peripheral edges of the coiled wire implants was found to be of a similar nature. Relative to the straight wire configurations and even to the peripheral edges of the coiled wire implants, increased cellularity was noted in the region between the coils. However, this result may be a consequence of the increased surface area and volume of space within the wire coils that promotes cellular growth and infiltration. Even at 4 weeks, this filling of the spaces within the lead by cellular material may still be in an active phase. In other longer term chronic implants of coiled wires, the capsule surrounding the wire, both peripherally and between the coils, was mature and characterized primarily by layers of fibroblasts and collagen.

Wire Implant Length

No significant difference in cellular response was found between the short and the long wire samples. The two wire lengths were employed in this study because of concern about the effects of micro-movement at the wire ends that might lead to an increase in inflammation. If that was the case, we might have expected to see an elevated cellular response in the shorter wire samples, where essentially all of the wire was within the vicinity of the wire ends. However, this was not seen.

Wire Implant Material

The primary objective of this study was to determine if an alternative insulation material, PFA fluoropolymer, warranted further investigation for use in our electrode leads. The evaluation was to be based on any observed differences between the cellular response to this alternative material and to the previously studied material, FEP fluoropolymer. Across all sample

groups, no overall difference in the cellular response to these two materials was observed. Slight differences in the response to the materials may have been masked by the relatively high degree of variability within sample groups. However, no consistent trends were noted between sample groups and no evidence of tissue necrosis was observed in any sample.

Conclusions

As evidenced by the cellular response from these studies, our alternative wire insulation material, PFA fluoropolymer, does warrant further investigation for use in our electrode leads. This material presents several advantages, in processing, availability and cost, that make it an attractive option to the previously studied FEP fluoropolymer. Based on these results, we feel comfortable using this material in other chronic animal implants and in our planned in vitro testing.

Encapsulation Tissue Response to Cuff Implants

The silicone rubber nerve cuffs were divided into 12 different implant groups, each with n=3, based on implant duration, implant material, and implant configuration (leads/no leads). The cuffs, fabricated without any electrode contacts, were placed on the sciatic nerve of each animal for durations of either 2 weeks or 4 weeks. The cuffs were made of three different silicone rubber formulations, Dow Corning's Silastic Q7-4550, NuSil's MED2-6640, and NuSil's MED2-6641-1. All of the cuffs were fabricated with a backbone of coiled lead wire incorporated within the cuff. In half of the cuffs, the leads were cut to be flush with the width of the cuff, while in the other half, the leads extended for 7-10 cm beyond the body of the cuff.

General Observations

As with the wire implants, a high degree of variability in cellular response was observed within the same group of cuffs and even within a single sample. The photomicrographs presented in the following figures demonstrate the range of cellular response observed across all cuff implant groups.

Cuff Implants: Range of Response

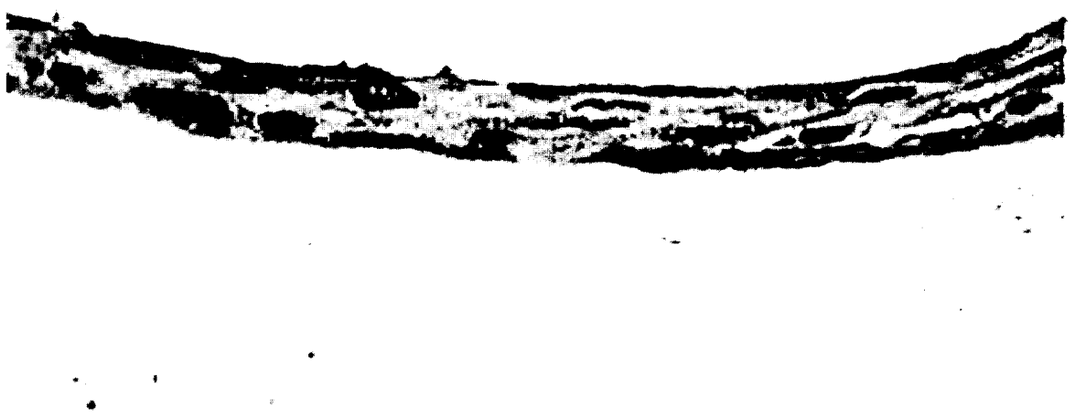

Figure 5: Photomicrograph of tissue capsule surrounding a cuff without leads made from NuSil's MED2-6640 and implanted for 4 weeks. (R2 #227 CT, H&E stain, original magnification=500x)

Figure 5 is an example of a very low level cellular response. The tissue in this case had developed between 2 layers of the cuff wraps, and was surrounded on both sides by the implant material. The capsule here consists of very few cell layers and is predominantly comprised of elongated fibroblasts, indicative of a stable response.

Cuff Implants: Range of Response

Figure 6: Photomicrograph of tissue capsule surrounding a cuff without leads made from Dow Corning's Silastic Q7-4550 and implanted for 4 weeks. (R3 #121 CT, H&E stain, original magnification=500x)

In Figure 6 is a relatively mild cellular response, where the capsule is seen to contain a few more cell layers than in the preceding figure, but still has only 1-2 layers of rounded active cells, with underlying layers of elongated fibroblasts and collagen.

Cuff Implants: Range of Response

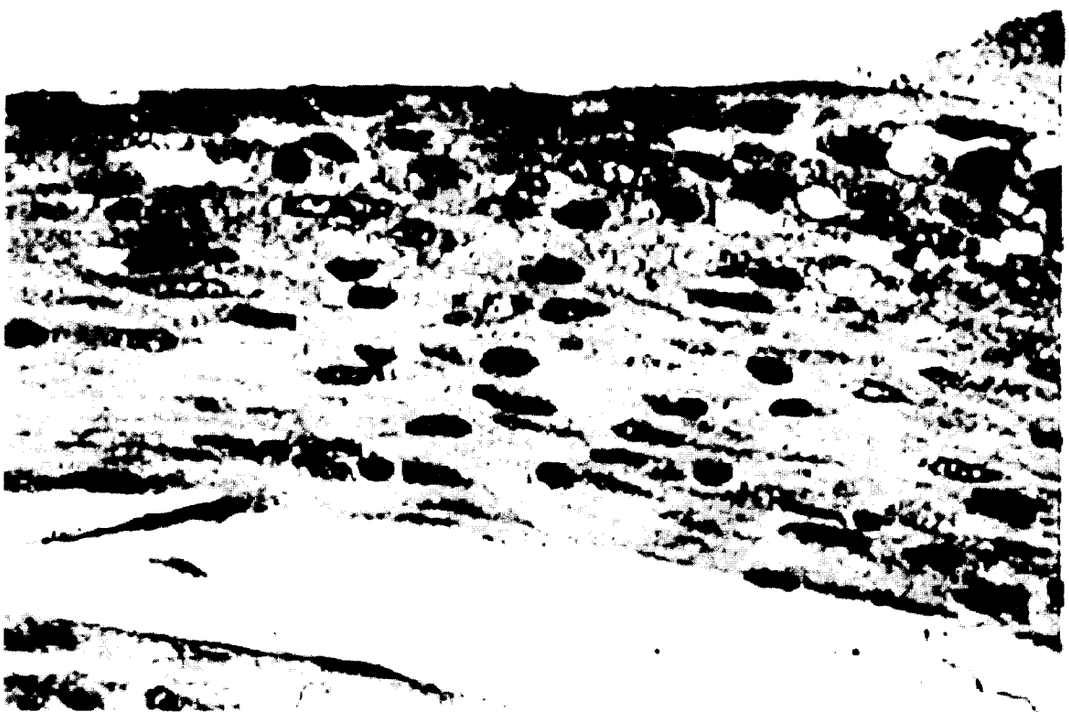

Figure 7: Photomicrograph of tissue capsule surrounding a cuff without leads made from NuSil's MED2-6640 and implanted for 4 weeks. (R2 #227 CT, H&E stain, original magnification=500x)

Figure 7 presents a slightly elevated cellular response. This is demonstrated by the increased numbers of rounded cells in the capsule adjacent to the implant material (top of figure), signaling an increase in metabolic activity. However, the periphery of the capsule is still characterized by multiple layers of elongated fibroblasts and collagen.

Cuff Implants: Range of Response

Figure 8: Photomicrograph of encapsulation tissue surrounding a cuff with leads made from NuSil's MED2-6641-1 and implanted for 4 weeks. (R15 #316 CT, H&E stain, original magnification=500x)

Figure 8 is an example of an even more elevated cellular response. The capsule here is characterized by almost exclusively active, rounded cells with very little organization, creating a loose cellular structure with few fibroblasts with collagen.

The photomicrographs presented in Figures 5-8 are representative of the range of cellular response found within each sample group. Two other general observations across sample groups, the presence of fat cells and increased cellularity in distal sections, are discussed briefly below.

In these samples, we observed frequent fat cells both around the nerve and within the capsule. In some cases, pockets of fat immediately surrounding the nerve prevented close contact between the cuff and the neural tissue. We have not noticed significant fat in previous studies of cuff electrodes that have been performed in cats. Whether the cat sciatic nerve is generally less fatty than normally found in other species or other nerves, whether slight pressure from the cuff electrode can cause resorption of fat over longer periods of time, or whether significant fat layers may affect the results of electrical stimulation are unresolved questions.

Also of note was a general increase in cellular response found in the more distal sections from these samples. This was observed even on a gross level during the tissue dissections, as in several samples the capsule was visibly more robust at the distal end relative to the proximal end. This was also true in the histological evaluation, where the capsule was oftentimes thicker and contained more cell layers in the distal segments.

Comparisons were made between corresponding sample groups to investigate any trends in cellularity and to determine the impact of differences in implant duration, configuration, and material. These results are summarized in the sections below.

Cuff Implant Duration

Again, consistent with expectations, we found a decrease in cellular response in those samples implanted for the longer time duration. This was demonstrated by the improved cellular organization, decreased numbers of round inflammatory cells, and general maturation of the capsule tissue.

Cuff Implant Configuration

Half of these cuffs contained a short extension of lead cable, which was included in an effort to determine whether the presence of a lead affected either the cellular response surrounding the implant, or through mechanical means, affected the neural response to the cuff. In analyzing the first of these, no notable difference was found in the cellular response to cuffs with or without leads.

Cuff Implant Material

The primary objective of this part of the study was to determine whether alternative silicone rubber formulations warranted further investigation for use in our nerve cuff electrodes. Although all sample groups elicited the range of cellular responses presented in the previous figures, samples of both NuSil materials tended to elicit a milder cellular response than that observed in the Dow samples. Between the two NuSil materials, while very little difference was found, the MED2-6640 implants

typically generated a decreased cellular response compared to the MED2-6641-1 implants. Based on these results, we feel that the NuSil silicone rubber formulations do warrant continued investigation for use in our cuff electrodes and we feel comfortable proceeding with additional chronic animal implants and in vitro testing of these materials.

Conclusions: Encapsulation Tissue Response

The primary objectives of this study were to determine whether we should continue our investigations of alternate fluoropolymer wire insulation and silicone rubber sheeting materials for use in our electrode systems. While previous handling and testing of the materials indicated that the original and replacement materials were comparable in both physical and mechanical properties, we had no data on their relative biocompatibility. These studies were not intended to provide quantitative evidence of the expected cellular response to long-term implants of these materials. Rather, we wanted to have a relative measure of the cellular response to these materials as compared to those previously used. The results from these studies show that both fluoropolymers generate a comparable level of inflammatory response. Additionally, the cellular response to our alternative silicone rubber formulations was comparable and even improved relative to the original silicone rubber formulation.

Future Work: Neural Tissue Response

While this study was intended primarily to compare the encapsulation tissue response to these materials, it provided an opportunity for us to investigate the neural tissue response as well. The bulk of our previous chronic testing of nerve cuffs has involved implant durations of 3-6 months, considerably longer than the 2 and 4 week implant durations of this study. Additionally, we speculated that the mechanical properties of the sheeting material and the lead cable may have an effect on the neural response to the implant. The neural tissue has been processed and preliminary evaluations have been made. In some samples, light microscopy evaluations were inconclusive, therefore, electron microscopy is being pursued. Specimens for transmission electron microscopy (TEM) have been prepared and TEM evaluation is currently underway. Because the results of the TEM analysis will affect our overall conclusions and comparisons between sample groups, the neural tissue response will be presented in a future progress report.